Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer

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Abstract

The last 7 years have seen over seven times as many publications indexed by Medline dealing with pomegranate and Punica granatum than in all the years preceding them. Because of this, and the virtual explosion of interest in pomegranate as a medicinal and nutritional product that has followed, this review is accordingly launched. The pomegranate tree, Punica granatum, especially its fruit, possesses a vast ethnomedical history and represents a phytochemical reservoir of heuristic medicinal value. The tree/fruit can be divided into several anatomical compartments: (1) seed, (2) juice, (3) peel, (4) leaf, (5) flower, (6) bark, and (7) roots, each of which has interesting pharmacologic activity. Juice and peels, for example, possess potent antioxidant properties, while juice, peel and oil are all weakly estrogenic and heuristically of interest for the treatment of menopausal symptoms and sequelae. The use of juice, peel and oil have also been shown to possess anticancer activities, including interference with tumor cell proliferation, cell cycle, invasion and angiogenesis. These may be associated with plant based anti-inflammatory effects, The phytochemistry and pharmacological actions of all Punica granatum components suggest a wide range of clinical applications for the treatment and prevention of cancer, as well as other diseases where chronic inflammation is believed to play an essential etiologic role.

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Keywords: Punica granatum; Cancer; Eicosanoid; Inflammation; Pomegranate

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Abbreviations: AA, arachidonic acid; ABTS, 2,2-azinobis(3-ethylbenzathiazoline-6-sulfonic acid); Akt, (protein kinase B); AOM, azoxymethane; CA, carbonic anhydrase; CAI, carbonic anhydrase inhibitor; COX, cyclooxygenase; cdK, cyclin dependent kinase; DMBA, 7,12-dimethyl-benz[a]anthracene; DMPD, N,N-dimethyl-p-phenylendiamine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EA, ellagic acid; FAS, a death receptor ligand; FRAP, ferric reducing antioxidant potency; HAEC, human aorta endothelial cells; HDL, high density lipoprotein; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxy-9,11-octadecadienoic acid; HPLC, high pressure liquid chromatography; IL, interleukin; J, ethyl acetate extract of fresh pomegranate juice; LDL, low density lipoprotein; LFA, leukotriene A; LTB, leukotriene B; LOX, lipoxygenase; MAPK, mitogen-activated protein kinase; MIF, migration inhibitory factor; MMOC, mouse mammary organ culture; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa B; NO, nitric oxide; NOS, nitric oxide synthase; NSAID, non-steroidal anti-inflammatory drug; O, polyphenol fraction of PSO; ODC, ornithine decarboxylase; P, pomegranate peel extract; PFE, pomegranate flower extract; PGD, prostaglandin D; PGE, prostaglandin E; PGF, prostaglandin F; PGG, prostaglandin G; PH, prostaglandin H; PGI, prostaglandin I; PGI2, prostacyclin; PJ, pomegranate juice; PSA, prostate specific antigen; PSE, aqueous/ethanolic pomegranate seed extract; PSO, pomegranate seed oil; ROS, reactive oxygen species; SESCO, supercritical CO2-extracted pomegranate seed oil; TEAC, Trolox equivalent antioxidant capacity; TPA, 12-O-tetradecanoylphorbol 13-acetate; TPT, total pomegranate tannin; TXA, thromboxane A; UV-b, ultraviolet b; VEGF, vascular endothelial growth factor; W, fermented pomegranate juice; WPFE, acetone extract of whole pomegranate fruits

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1. Introduction

It has been 8 years since we published in this Journal the first report of antioxidant and anti-inflammatory actions of pomegranate fractions in vitro. Medline now cites 138 new scientific papers relating to health effects of pomegranate, compared to only 25 between 1950 and summer 1999. Many of these new papers have focused on antioxidant actions in vitro, ex vivo and in vivo, while other work has elaborated on the ability of pomegranate juice, seed oil, peel or flower extracts, and their derivatives to kill bacteria and viruses, or to fight vascular disease, diabetes and cancer. In everything from improving erectile insufficiency in rabbits to healing ethanol induced stomach ulcers in rats, antioxidant action is given as the leitmotif and root of the observed beneficial effects. Driven by such studies, sales of pomegranate juice are soaring worldwide, with even pomegranate seed oil beginning to appear in the marketplace.

The enthusiasm for health effects of pomegranate, however, may be only partially justified. For one thing, the straight line that many ascribe between in vitro, in silico and even in vivo antioxidant effects on the one hand, and protection against neurological damage, ulcers, high cholesterol, cancer, arterial plaques and impotence on the other, has not been solidly established. Although redox status may serve as a trigger of inflammation-modulating cytokines (Flohe et al., 1997) or of angiogenesis (Rojas et al., 2006), its precise role in regulating disease is still unclear. Secondly, research into the effects of pomegranate derivatives on human health is still at a very early stage. Few well controlled clinical trials have yet been completed, and the toxicology of pomegranate fractions, particularly their potential for mutagenesis, is only now beginning to be addressed.

Much deeper investigation into this rapidly growing field is thus required to assess the overall value and safety of pomegranate as an intact fruit or of various extracts derived from pomegranate components. In order to facilitate such research, the present review is needed and accordingly, offered.

Punica granatum (Fig. 1) shares its botanical family only with Punica protopunica, the latter restricted in occurrence to Socotra, an island off the Yemeni coast. Over 1000 cultivars of Punica granatum exist (Levin, 1994), originating from the Middle East, extending throughout the Mediterranean, eastward to China and India, and on to the American Southwest, California and Mexico in the New World. While the pomegranate plant is considered either a small tree or a large shrub, its fruit is often deemed to be a large berry. The fruit is delimited by a leathery pericarp, contained within are numerous arils, each a single seed surrounded by a translucent juice-containing sac. Thin acrid-tasting membranes extend into the interior of the fruit from the pericarp, providing a latticework for suspending the arils. Thus, the fruit itself gives rise to three parts: the seeds, about 3% of the weight of the fruit, and the interior of the fruit from the pericarp, providing a latticework for suspending the arils. Thus, the fruit itself gives rise to three parts: the seeds, about 30% of the weight of the fruit, and themselves containing about 20% oil, the juice, about 30% of the fruit weight, and the peels (pericarp) which also include the interior network of membranes. Other useful parts of the plant include the roots, bark, leaves, and flowers.

The history of pomegranates with respect to development of mankind is impressive. An 800-year old Kabbalistic text, Sefer ha Rimon: The Book of the Pomegranate, equates pomegranate with Shekinah, the female aspect of Creation, and Its Creator (Wolfson, 1988). Pomegranates feature prominently in Judaism, Christianity, Islam, Buddhism and Zoroastrianism. Pomegranates appear in the coats of arms of several British med-
flowers serve as a remedy for diabetes mellitus (Saxena and Vikram, 2004). Modern uses of pomegranate derived products now include treatment of acquired immune deficiency syndrome (AIDS) (Lee and Watson, 1998), in addition to use for cosmetic beautification (Kawamada and Shimada, 2002; Moayadi, 2004) and enhancement (Curry, 2004), hormone replacement therapy (Lansky, 2000), resolution of allergic symptoms (Watanabe and Hatakoshi, 2002), cardiovascular protection (Shiraishi et al., 2002; Aviram and Dornfeld, 2003), oral hygiene (Kim and Kim, 2002), ophthalmic ointment (Bruijn et al., 2003), weight loss soap (Guojian, 1995), and as an adjunct therapy to increase bioavailability of radioactive dyes during diagnostic imaging (Il’iasov, 1975; Amorim et al., 2003).

Over the past few decades scientific investigations have laid a credible basis for some of the traditional ethnomedical uses of the pomegranate. These studies, most completed in the past 5 years, may be divided into several general areas. For example, pomegranate mediated antioxidant activity can be considered a means of lowering the threshold for inflammation. Antioxidant activity, as well as suppression of inflammation, may contribute to chemotherapeutic and chemopreventive utility against cancer. Investigations of the pharmacology and health benefits claimed from use of pomegranate components to these three broad, but interconnected areas (antioxidant, anti-inflammatory and anti-cancer) as well as an introduction to the chemical constituents of Punica granatum, will be discussed in this review.

2. Chemistry

While detailed knowledge of relationships of the chemical content of pomegranates and their desirable pharmacologic endpoints has yet to be obtained, significant progress has been made over the past 8 years toward a much more comprehensive understanding of some of the important pharmacologic components of pomegranate. These are summarized, with their structures, in Table 1. In addition to the more common anthocyanins shown in the table, pentose glycosides of malvidine and pentunidin have been described in the pericarp and juice (Sharma and Seshadri, 2002). A selected list of compounds is shown in Table 1. In addition to the more common anthocyanins shown in the table, pentose glycosides of malvidine and pentunidin have been described in the pericarp and juice (Sharma and Seshadri, 2002). A selected list of compounds is shown in Table 1. In addition to the more common anthocyanins shown in the table, pentose glycosides of malvidine and pentunidin have been described in the pericarp and juice (Sharma and Seshadri, 2002).

2.1. Seed

Pomegranate seed oil (PSO) comprises 12–20% of total seed weight. The oil consists of approximately 80% conjugated...
Table 1
Selected compounds of *Punica granatum*

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Compound name</th>
<th>Compound structure</th>
<th>Plant part: J: juice, P: leaf, F: flower, L: leaf, S: seed, B: bark of tree, R: bark of tree root</th>
<th>References</th>
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<tbody>
<tr>
<td>Simple sugars</td>
<td>Glucose</td>
<td>J</td>
<td>Cui et al. (2004)</td>
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<tr>
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<td>Fructose</td>
<td>J</td>
<td>Cui et al. (2004)</td>
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<td>Sucrose</td>
<td>J</td>
<td>Gabbasova and Abdurazakova (1969)</td>
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<td>Citric acid</td>
<td>J</td>
<td>Poyrazoglu et al. (2002)</td>
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<td>their salts</td>
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<td>Cyclitol carboxylic acids and</td>
<td>Brevifolin carboxylic acid 10-monopotassium sulphate</td>
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<td>Hussein et al. (1997)</td>
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<td><img src="image" alt="Rutin Structure" /></td>
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<td>1,2,3-Tri-O-galloyl-β-D-glucose</td>
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<td>L</td>
<td>Nawwar et al. (1994b)</td>
</tr>
<tr>
<td>Ellagitannins</td>
<td>Punicacortein A</td>
<td><img src="image5" alt="Compound Structure" /></td>
<td>B, R</td>
<td>Tanaka et al. (1986b)</td>
</tr>
<tr>
<td>Ellagitannins</td>
<td>Punicacortein B</td>
<td><img src="image6" alt="Compound Structure" /></td>
<td>B, R</td>
<td>Tanaka et al. (1986b)</td>
</tr>
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Table 1 (Continued)

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<tbody>
<tr>
<td>Ellagitannins</td>
<td>Punicaortein C</td>
<td><img src="image1" alt="Compound Structure" /></td>
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<td>Tanaka et al. (1986b)</td>
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<tr>
<td>Ellagitannins</td>
<td>Punicaortein D</td>
<td><img src="image2" alt="Compound Structure" /></td>
<td>B, R</td>
<td>Tanaka et al. (1986b)</td>
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<td>Ellagitannins</td>
<td>Punigluconin 2,3-di-O-galloyl-4,6-(S)-hexahydroxydiphenoylglutonic acid</td>
<td><img src="image3" alt="Compound Structure" /></td>
<td>B, R</td>
<td>Tanaka et al. (1986b)</td>
</tr>
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<td>Amino acids</td>
<td>Proline</td>
<td><img src="image4" alt="Amino Acid Structure" /></td>
<td>J</td>
<td>Velioglu et al. (1997)</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Valine</td>
<td><img src="image5" alt="Amino Acid Structure" /></td>
<td>J</td>
<td>Seppi Ak Franciosi (1980)</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Methionine</td>
<td><img src="image6" alt="Amino Acid Structure" /></td>
<td>J</td>
<td>Seppi Ak Franciosi (1980)</td>
</tr>
<tr>
<td>Indoleamines</td>
<td>Tryptamine</td>
<td><img src="image7" alt="Indoleamine Structure" /></td>
<td>J</td>
<td>Badria (2002)</td>
</tr>
<tr>
<td>Indoleamines</td>
<td>Serotonin</td>
<td><img src="image8" alt="Indoleamine Structure" /></td>
<td>J</td>
<td>Badria (2002)</td>
</tr>
<tr>
<td>Indoleamines</td>
<td>Melatonin</td>
<td><img src="image9" alt="Indoleamine Structure" /></td>
<td>J</td>
<td>Badria (2002)</td>
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Table 1 (Continued)

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<tbody>
<tr>
<td>Pelletierine alkaloids</td>
<td>N-Methylpelletierene</td>
<td></td>
<td>B, R</td>
<td>Neuhofer et al. (1993)</td>
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<tr>
<td>Pelletierine alkaloids</td>
<td>Pseudopelletierene</td>
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<td>B, R</td>
<td>Neuhofer et al. (1993)</td>
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<td>Pelletierine alkaloids</td>
<td>Norpseudopelletierene</td>
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<td>R</td>
<td>Neuhofer et al. (1993)</td>
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<td>Piperidine alkaloids</td>
<td>Sedridine</td>
<td>R</td>
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<td>Neuhofer et al. (1993)</td>
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<tr>
<td>Piperidine alkaloids</td>
<td>2-(2′-Hydroxypropyl)Δ1- piperideine</td>
<td>R</td>
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<td>Neuhofer et al. (1993)</td>
</tr>
<tr>
<td>Piperidine alkaloids</td>
<td>2-(2′-Propenyl)Δ1- piperideine</td>
<td>R</td>
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<td>Neuhofer et al. (1993)</td>
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<td>Piperidine alkaloids</td>
<td>N-(2′-5′-Dihydroxyphenyl) pyridium chloride</td>
<td>L</td>
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<td>Pyrrolidine alkaloid</td>
<td>Hygrine</td>
<td>R</td>
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<td>Neuhofer et al. (1993)</td>
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<tr>
<td>Pyrrolidine alkaloid</td>
<td>Norhygrine</td>
<td>R</td>
<td></td>
<td>Neuhofer et al. (1993)</td>
</tr>
<tr>
<td>Conjugated fatty acids</td>
<td>Punicic acid (cis-9, trans-11, cis-13 octadecatrienoic acid)</td>
<td>S</td>
<td></td>
<td>Schubert et al. (1999)</td>
</tr>
<tr>
<td>Non-conjugated fatty acids</td>
<td>Linoleic acid</td>
<td>S</td>
<td></td>
<td>Hopkins and Chisholm (1968), Schubert et al. (1999), Hornung et al. (2002)</td>
</tr>
<tr>
<td>Non-conjugated fatty acids</td>
<td>Oleic acid</td>
<td>S</td>
<td></td>
<td>Schubert et al. (1999)</td>
</tr>
<tr>
<td>Non-conjugated fatty acids</td>
<td>Palmitic acid</td>
<td>S</td>
<td></td>
<td>Schubert et al. (1999)</td>
</tr>
<tr>
<td>Non-conjugated fatty acids</td>
<td>Stearic acid</td>
<td>S</td>
<td></td>
<td>Schubert et al. (1999)</td>
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<tr>
<td>Sterols</td>
<td>Daucosterol</td>
<td><img src="image" alt="Daucosterol structure" /></td>
<td>S</td>
<td>Wang et al. (2004)</td>
</tr>
<tr>
<td>Sterols</td>
<td>Camesterol</td>
<td><img src="image" alt="Camesterol structure" /></td>
<td>S</td>
<td>Abd El Wahab et al. (1998)</td>
</tr>
<tr>
<td>Sterols</td>
<td>Stigmasterol</td>
<td><img src="image" alt="Stigmasterol structure" /></td>
<td>S</td>
<td>Abd El Wahab et al. (1998)</td>
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<tr>
<td>Sterols</td>
<td>β-Sitosterol</td>
<td><img src="image" alt="β-Sitosterol structure" /></td>
<td>S</td>
<td>Abd El Wahab et al. (1998)</td>
</tr>
<tr>
<td>Sterols</td>
<td>Cholesterol</td>
<td><img src="image" alt="Cholesterol structure" /></td>
<td>S</td>
<td>Abd El Wahab et al. (1998)</td>
</tr>
<tr>
<td>Sex steroids</td>
<td>17-α-Estradiol</td>
<td><img src="image" alt="17-α-Estradiol structure" /></td>
<td>S</td>
<td>Kim et al. (2002), Lansky et al. (2005a)</td>
</tr>
<tr>
<td>Sex steroids</td>
<td>Estrone</td>
<td><img src="image" alt="Estrone structure" /></td>
<td>S</td>
<td>Heftmann et al. (1966), Dean et al. (1971), Abd El Wahab et al. (1998)</td>
</tr>
<tr>
<td>Sex steroids</td>
<td>Testosterone</td>
<td><img src="image" alt="Testosterone structure" /></td>
<td>S</td>
<td>Abd El Wahab et al. (1998)</td>
</tr>
<tr>
<td>Sex steroids</td>
<td>Estriol</td>
<td><img src="image" alt="Estriol structure" /></td>
<td>S</td>
<td>Abd El Wahab et al. (1998)</td>
</tr>
<tr>
<td>Tocopherols</td>
<td>γ-Tocopherol</td>
<td><img src="image" alt="γ-Tocopherol structure" /></td>
<td>S</td>
<td>Kim et al. (2002)</td>
</tr>
</tbody>
</table>
Table 1 (Continued)

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</thead>
<tbody>
<tr>
<td>Triterpenoids</td>
<td>Ursolic acid</td>
<td><img src="image" alt="Ursolic acid structure" /></td>
<td>S, F</td>
<td>Ahmed et al. (1995), Huang et al. (2005c)</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Oleanolic acid</td>
<td><img src="image" alt="Oleanolic acid structure" /></td>
<td>F</td>
<td>Huang et al. (2005a)</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Maslinic acid</td>
<td><img src="image" alt="Maslinic acid structure" /></td>
<td>F</td>
<td>Batta and Rangaswami (1973)</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Asiatic acid</td>
<td><img src="image" alt="Asiatic acid structure" /></td>
<td>F</td>
<td>Batta and Rangaswami (1973)</td>
</tr>
<tr>
<td>Glycolipids</td>
<td>Cerebroside</td>
<td><img src="image" alt="Cerebroside structure" /></td>
<td>S</td>
<td>Tsuyuki et al. (1981)</td>
</tr>
<tr>
<td>Coumestan</td>
<td>Coumestrol</td>
<td><img src="image" alt="Coumestrol structure" /></td>
<td>S</td>
<td>Moneam et al. (1988), Micheli et al. (1962)</td>
</tr>
<tr>
<td>Phenyl aliphatic glycosides</td>
<td>Coniferyl 9-O-β-D-apiofuranosyl(1→6)-O-β-D-glucopyranoside</td>
<td><img src="image" alt="Coniferyl structure" /></td>
<td>S</td>
<td>Wang et al. (2004)</td>
</tr>
</tbody>
</table>
A. Phenyl aliphatic glycosides

2.2. Juice

Anthocyanins, potent antioxidant flavonoids, provide pomegranate juice with its brilliant color, which increases in intensity during ripening (Hernandez et al., 1999), and declines after pressing (Perez-Vicente et al., 2002; Miguel et al., 2004). Minerals in the juice and seed include Fe, relatively prevalent, but not in so high concentrations as in watermelon, and Ca, Ce, Cl, Co, Cr, Cs, Cu, K, Mg, Mn, Mo, Na, Rb, Sc, Se, Sn, Sr, and Zn (Waheed et al., 2004).

2.3. Pericarp (peel, rind, hull are synonyms)

Both flavonoids and tannins are more abundant in the peels of wild-crafted compared to cultivated fruits (Ozcal and Dinc, 1993). Complex polysaccharides from the peels have been studied and partially characterized (Jahfar et al., 2003). The presence of alkaloids (e.g., pelletierine) in the peel is equivocal, positive by Dragendorff assay, but negative by Mayer assay (Vidal et al., 2003).

2.4. Leaf

Unique tannins occur in pomegranate leaves, as well in peel. Leaves also contain glycosides of apigenin, a flavone with progestinic (Zand et al., 2000) and anxiolytic (Paladini et al., 1999) properties. With respect to chemical elements, N is high in medium age, K in young age; Ca and Fe in old leaves. In July and August in the Northern Hemisphere, N and K are both low during flowering and fruit-setting, N further declines during fruit maturity, along with Mg, Fe and Zn (Munde et al., 1980, 1981).

2.5. Flower

The flowers contain compounds also found in peels (e.g. gallic acid) and seed (e.g. ursolic acid), and quite possibly unique, distinctive compounds as well (Huang et al., 2005c). Further study is in process to elucidate the chemistry of these flowers that have also been ethnomedically employed.

2.6. Tree bark and roots

Extracts prepared from the rougher parts of the tree also have potent physiological effects and a long medical history. Their chemistry is notable against that of other tree parts mainly for the extensive presence of alkaloids.

Table 2 highlights of some of the major chemical components of pomegranate seeds, juice, pericarp, bark and leaf, and their pharmacologic activity in mammalian cells relevant to the prevention and/or treatment of malignant cell growth. While multiple mechanisms reflect the fruit’s chemical complexity, major themes of increased apoptosis, decreased inflammation, decreased metastasis and invasion, as well as a decrease in drug

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</tr>
</thead>
<tbody>
<tr>
<td>Phenyl aliphatic glycosides</td>
<td>Sinapyl 9-O-[[β-D-apiofuranosyl(1→6)]-O-β-D-glucopyranoside</td>
<td>S</td>
<td>Wang et al. (2004)</td>
<td></td>
</tr>
<tr>
<td>Phenyl aliphatic glycosides</td>
<td>Phenethyl rutinoside</td>
<td>S</td>
<td>Wang et al. (2004)</td>
<td></td>
</tr>
<tr>
<td>Phenyl aliphatic glycosides</td>
<td>Icariside D1</td>
<td>S</td>
<td>Wang et al. (2004)</td>
<td></td>
</tr>
</tbody>
</table>

octadecatrienic fatty acids, with a high content of cis 9, trans 11, cis 13 acid (i.e. punicic acid), synthesized in situ from non-conjugated octadecadienoic fatty acid, linoleic acid (Hopkins and Chisholm, 1968; Hornung et al., 2002), itself about 7% of PSO. The fatty acid component of PSO comprises over 95% of the oil, of which 99% is triacylglycerols. Minor components of the oil include sterols, steroids, and a key component of mammalian myelin sheaths, cerebroside (Tsuyuki et al., 1981). Seed matrix includes lignins (Dalimov et al., 2003), fusion products of cell wall components and hydroxycinnamic acids, and potently antioxidant lignin derivatives (Wang et al., 2004).
Table 2
Key compounds in pomegranate and their known anticancer or anti-inflammatory 1 actions

<table>
<thead>
<tr>
<th>Plant component</th>
<th>Compounds or compound classes with known anti-cancer or anti-inflammatory effects</th>
<th>Therapeutic activities of relevance to inflammation, cancer prevention and treatment</th>
<th>Recent references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>Ursolic acid</td>
<td>Apoptosis in endometrial cancer cells via caspase 3 pathway</td>
<td>Achiwa et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Sterols (daucosterol, campesterol, stigmasterol, beta-sitosterol)</td>
<td>Apoptosis in melanoma cells via the intrinsic cell death pathway and caspase 3 activation</td>
<td>Harmand et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Punicic acid</td>
<td>Inhibits PC-3 invasion</td>
<td>Lansky et al. (2005b)</td>
</tr>
<tr>
<td>Juice and peels</td>
<td>Hydroxybenzoic acids (gallic and ellagic)</td>
<td>Induces p53/p21 expression, G1 arrest and apoptosis in bladder cancer cells</td>
<td>Li et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Hydroxycinnamic acids (e.g., caffeic)</td>
<td>Causes growth inhibition and apoptotic death of human DU-145 prostate cancer cells</td>
<td>Veluri et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Catechins and epicatechins</td>
<td>Antagonize growth-factor induced proliferative diseases</td>
<td>Doss et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Proanthocyanidins and anthocyanidins</td>
<td>Antiangiogenic, antioxidant and anticarcinogenic activities</td>
<td>Bagchi et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>Antitumor effects of flavonoids (review)</td>
<td>Kanadaswami et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Ellagitannins (punicalin and punicalagin)</td>
<td>UV-B mediated activation of NF-κB</td>
<td>Afaq et al. (2005a)</td>
</tr>
<tr>
<td></td>
<td>Flavonols (e.g., kaempferol)</td>
<td>Expression of tumor necrosis factor-α, interleukin-1β gene expression in tumor cells</td>
<td>Kowalski et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Flavones (e.g., luteolin)</td>
<td>Antiproliferative, apoptotic and antioxidant activities</td>
<td>Seeram et al. (2005)</td>
</tr>
</tbody>
</table>

In addition to the polyphenolic compounds found in juice.

Peels

Flavonols (e.g., kaempferol)

Acts synergistically with quercetin to inhibit breast cancer cell proliferation

 PUl- mediated activation of NF-κB

Malignant-activated protein kinases (MAPK)

Inflammatory cell signaling in colon cancer

Antiproliferative, apoptotic and antioxidant activities

Flavones (e.g., luteolin)

 Expression of tumor necrosis factor-α, interleukin-1β gene expression in tumor cells

Inhibition of lung cancer cell growth via G2/M arrest and induction of apoptosis

↓ Expression of tumor necrosis factor-α, interleukin-1β gene expression in tumor cells

↓ Expression of tumor necrosis factor-α, interleukin-1β gene expression in tumor cells

↓ Expression of tumor necrosis factor-α, interleukin-1β gene expression in tumor cells

↓ Expression of tumor necrosis factor-α, interleukin-1β gene expression in tumor cells

↑ Fatty acid synthase activity in human tumor cells

↑ Focal adhesion kinase activity, a key regulator of tumor cell invasion

↑ Tumor cell apoptosis through up-regulation of death receptor and activation of caspase activities

↑ Tumor cell apoptosis through up-regulation of death receptor and activation of caspase activities

↑ Tumor cell apoptosis through up-regulation of death receptor and activation of caspase activities

↑ Tumor cell apoptosis through up-regulation of death receptor and activation of caspase activities
resistance, are evident. For example, compounds like ursolic acid, γ-tocopherol, ellagic acid, quercetin, ellagitannins, luteolin and apigenin have all been associated with tumor cell apoptosis. This is achieved through a decline in activation of NF-κB, a decrease in fatty acid synthase activity and tumor necrosis factor, increased caspase activities and upregulation of p21 and p53 expression. Pomegranate component control of inflammation involves inhibition of both COX and LOX by conventional non-steroidal anti-inflammatory drugs (NSAID's) may adversely affect cardiovascular function (Grosser et al., 2006) due to suppression of PGI2 (prostacyclin), a prostanoid required for cardiovascular homeostasis that prevents platelet aggregation, induces vasodilation and down-regulates expression of endothelial cell adhesion molecules (Noguchi et al., 2000). Interestingly, the opposite appears true for pomegranate juice (PJ). Compared to orange juice, purple grape juice (PGJ), and coffee, PJ most potently promotes PGJ expression in human subjects 20 min and six hours after consumption, though PGJ more strongly promoted factors (e.g. NF-kB), and bioactive lipids such as eicosanoids (e.g. prostaglandin E2 and lipoxygenase derived products). Elucidation of these complex inflammation-to-cancer mechanisms suggests new cancer prevention and therapeutic strategies, with pomegranate a comparably complex and intriguing potential source for the strategic agents.

3.1. Eicosanoid enzyme inhibition (Fig. 2)
Punic acid (Nugteren and Christ-Hazelhof, 1987) and polyphenols (Landolfi et al., 1984; Welton et al., 1986; Wallace, 2002; Morikawa et al., 2003) inhibit prostaglandin biosynthesis. The ethyl acetate extract of pomegranate fermented juice (W) inhibits soybean lipoxygenase (LOX) but not sheep cyclooxygenase (COX), while, a phenolic-rich extract of pomegranate seed oil (O) strongly inhibits lipoxygenase and cyclooxygenase (Schubert et al., 1999). Applied to mouse skin, whole pomegranate aqueous extract inhibits cyclooxygenase expression (Afaq et al., 2005b), while W, pomegranate peel extract (P) and pomegranate seed oil (PSO) each inhibit human PC-3 human prostate cancer cell phospholipase A2 expression in vitro. These suppressive effects are supra-additively enhanced when two of W, P and PSO, but especially when all three pomegranate components are combined (Lansky et al., 2005a).

Inhibition of COX by conventional non-steroidal anti-inflammatory drugs (NSAID’s) may adversely affect cardiovascular function (Grosser et al., 2006) due to suppression of PGI2 (prostacyclin), a prostanoid required for cardiovascular homeostasis that prevents platelet aggregation, induces vasodilation and down-regulates expression of endothelial cell adhesion molecules (Noguchi et al., 2000). Interestingly, the opposite appears true for pomegranate juice (PJ). Compared to orange juice, purple grape juice (PGJ), and coffee, PJ most potently promotes PGJ expression in human subjects 20 min and six hours after consumption, though PGJ more strongly promoted
Fig. 2. Simplified diagram of eicosanoid metabolism. Phospholipase A2 (PLA2) acts upon the cell membrane phospholipids bilayer to liberate arachidonic acid (AA) into the cell where it serves as a substrate for a variety of enzymes. Alternatively, other compounds such as linoleic acid typically derived from the diet can also serve as substrates for eicosanoid enzymes but result in different products. Prostaglandin E2 (PGE2) may be converted by cyclooxygenase 2 (COX-2) from AA, becoming an important stimulus for tumor cell proliferation, while AA can also be converted to lipoxygenase (LOX)-derived products, many of which as well stimulate tumor cell proliferation. These products include 12-hydroxyeicosatetraenoic acid (12-HETE) and 5-hydroxyeicosatetraenoic acid (5-HETE) derived from 12-LOX and 5-LOX, respectively. Other eicosanoid products have been shown to be involved in control of tumor cell proliferation. Such include 13-hydroxy-9,11-octadecadienoic acid (13-S-HODE) and 15-HETE although the function of these eicosanoids depends in part on the tumor cell environment in which they are generated. Punicic acid mediated inhibition of 5-LOX action has been established (unpublished data, not shown), and other points of modulation of both eicosanoid enzyme activities and expression are indicated by the red boxes. Note PGI2 expression is promoted by PJ, though inhibited by conventional NSAID’s. For more details, see text.

PGI2 expression two hours post-drinking, but only PJ promoted PGI2 synthesis in human aorta endothelial cells (HAEC) in vitro (Polagruto et al., 2003).

3.2. Cytokines

Harmful influences such as UV-b radiation may provoke DNA strand breaks, resulting in changes in phosphorylation status of proteins (Halicka et al., 2005). When such proteins are pro-inflammatory cytokines (biological response modifiers), protein modifications induce inflammatory cascades. Thus, investigation of these cascades is continuing to provide possible pharmaceutical targets, since chronic inflammation can serve as an important etiologic factor for chronic diseases including cancer (Aggarwal and Shishodia, 2004; Dominguez et al., 2005). Of interest, therefore, is the finding that acetone extracts of whole pomegranate fruits (WPFE) inhibited phosphorylation of several such cytokines in UV-B irradiated keratinocytes, including mitogen activated protein kinases (MAPK). The extracts also diminished activation of NF-κB (Afaq et al., 2005a). Inhibition of NF-κB, MAPK and related cytokines by WPFE occurred in vivo in mouse skin exposed to 12-O-tetradecanoylphorbol-13-acetate (TPA) (Afaq et al., 2005b), and in human chondrocytes induced by cytokine interleukin IL-1β (Ahmed et al., 2005) with up-regulation of MAPK-APK2 in PSO-treated human DU-145 prostate cancer cells (Albrecht et al., 2004). The beneficial effect of pomegranate extract reduction of cytokine activity has been shown to occur in patients with periodontitis. Patients experiencing this form of oral inflammation received intraviginal chips impregnated with pomegranate peel extract (and extract of Centella asiatica), which resulted in reduced inflammatory cytokines (IL-1beta and IL-6) several months post-treatment (Sastravaha et al., 2005).

3.3. Eicosanoid/cytokine cross talk

Pure punicalagin, “total pomegranate tannin extract” (TPT) or PJ, the latter two each containing 1.74 g punicalagin/L, all significantly and dose-dependently inhibited TNF-α induced COX-2 expression (associated with cell proliferation) in HT-29 human colon cancer cells. The PJ was much more potent than the TPT which in turn was more potent than pure punicalagin, although the amount of punicalagin in PJ was only 1/300th the amount used either alone or in the TPT. Decreased phosphorylation of the p65 subunit and decreased binding to the NF-κB response element were effected as TPT > PJ > punicalagin, and
ellagic acid (EA) was without effect. Also, PJ abolished TNFα-induced Akt (protein kinase B) activation (required for NF-κB activity), while EA and punicalagin were inactive (Adams et al., 2006). The components of PJ thus might appear to synergistically suppress inflammatory cytokine expression. Most recently, a whole pomegranate methanol extract was also shown to inhibit, in a dose-dependent manner, production and expression of TNFα in microglial cells, in which inflammation had been induced by lipopolysaccharide (Jung et al., 2006).

3.4. Matrix metalloproteinases

Matrix metalloproteinases (MMP) are enzymes important in maintenance of normal cellular architecture, assisting with creation of interstitial spaces by destroying structural proteins thereby facilitating multiple inflammatory processes (Shapiro, 1997; Leppert et al., 2001; Okamoto et al., 2004; Salvi and Lang, 2005). Human chondrocyte MMP’s were inhibited by WFPE (Ahmed et al., 2005), while W and to a lesser extent W and pomegranate seed extract (PSE), but not PSO, inhibited human dermal fibroblast MMP-1 (Aslam et al., 2005).

3.5. Antioxidant activity

Oxidative tension is a potent yet non-specific metabolic trigger for both inflammation and angiogenic processes (Hayden and Tyagi, 2004; Karageuzyan, 2005; Kapoor et al., 2005), both of which are key factors in cancer initiation and promotion (Dobrovolskaia and Kozlov, 2005; Garcea et al., 2005; Ohshima et al., 2005). Since pomegranate’s antioxidative efficacy clinically may be impaired by poor bioavailability of active compounds (Cerda et al., 2004, 2006), strengths and weaknesses of pomegranate’s antioxidant activity need be considered. In general, comparable juice or extracts from other common fruits show antioxidant activity in vitro inferior to that of the pomegranate (Halvorsen et al., 2002; Kelawala and Ananthanarayan, 2004; Xu et al., 2005).

Antioxidant activities associated with different pomegranate components are summarized in Table 3. In addition to these studies, other in vivo and clinical findings have been suggested as stemming from antioxidant effects. Examples of in vivo studies of beneficial effects of pomegranate antioxidant activity include: protection of rat gastric mucosa from ethanol or aspirin toxicity (Khennouf et al., 1999; Ajaikumar et al., 2005), protection of neonatal rat brain from hypoxia (Loren et al., 2005), prevention of male rabbit erectile tissue dysfunction (Azadzoi et al., 2005), and abrogation of ferric nitrolotrinacetate (Fe-NTA) induced hepatoxotoxicity evidenced by mitigated hepatic lipid peroxidation, actions of glutathione (GSH), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) glutathione-S-transferase (GST), serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), bilirubin and albumin levels, hepatic ballooning degeneration, fatty changes, and necrosis (Kaur et al., 2006). Cardiovascular effects of PJ in man, which may or may not involve redox-linked biochemical pathways, include lowering of LDL and total cholesterol (Esmailzadeh et al., 2004), ameliorating systolic hypertension (Aviram and Dornfeld, 2001), and reducing carotid arterial stenosis (Aviram et al., 2004).

4. Cancer prevention

4.1. Carcinogenesis

In mouse mammary organ culture (MMOC), an ex vivo model for pre-cancerous tumor initiation via exposure to chemical carcinogen 7,12-dimethyl-benz[a]anthracene (DMBA), W produced a 46% decrease in tumor occurrence (Kim et al., 2002), whereas cold-pressed PSO or an HPLC “Peak B” isolated from W resulted in up to an 87% reduction in tumor occurrence. Notably, 1 μg/ml PSO resulted in higher suppression than a 10 μg/ml dose (Mehta and Lansky, 2004) suggesting that an optimal biological dose is more important and relevant than a maximally tolerated one. In female CD-1 mice with skin tumors induced by DMBA and subsequently promoted by 12-O-tetradecanoylphorbol 13-acetate (TPA), external treatment with 5% PSO produced significant decreases in both tumor incidence and multiplicity (P < 0.05) (Hora et al., 2003). Similarly, topical pre-treatment with WPFE (2 mg/mouse) prior to TPA applications in DMBA-treated CD-1 mice decreased the tumor incidence from 100 to 30% and increased latency of tumor development from week 9 to week 14 (Afaq et al., 2005b). Pomegranate seed oil (PGO) has also been shown to reduce both the incidence and multiplicity of colon tumors in rats treated with carcinogen azoxymethane (AOM). As in MMOC, dose response is non-linear: there is an early peak, followed by a decline. The response pattern is as follows: for tumor incidence, AOM + 0.1% PGO, 44%, P < 0.05; AOM + 0.1% PGO, 38%, P < 0.01; AOM + 1% PGO, 56%) and for multiplicity: AOM + 0.1% PGO, 0.56 ± 0.73, P < 0.01; AOM + 0.1% PGO, 0.50 ± 0.73, P < 0.005; AOM + 1% PGO, 0.88 ± 0.96, P < 0.05 (Kohno et al., 2004). This study importantly establishes the chemopreventive activity of pomegranate seed oil against both tumor incidence and multiplicity, and also dramatically elaborates the non-linear character of these effects (i.e., a lower dose of oil may have a greater chemopreventive effect than a higher dose of oil). The study will serve as a springboard to future investigations seeking to establish optimum dosages of PSO for human cancer chemoprevention.

4.2. Cell cycle

Cell cycle changes occur following exposure of human Burkitt’s lymphoma cells to pomegranate peel extract (Settehetham and Ishida, 1995) and human monocyteic leukemia cells to pomegranate seed oil (Suzuki et al., 2001). Mechanisms for these effects likely involve modulation of cell signaling molecules in the cell cycle machinery (e.g., WAF1/p21) in the case of the pomegranate aqueous fractions (peel and juice) (Shukla and Gupta, 2004), and for PSO and its conjugated trienes, lipid peroxidation (Suzuki et al., 2001; Tsuzuki et al., 2004) and/or lipoygenase inhibition (Cunningham et al., 1997). Significant increases in DU-145 androgen negative human
Table 3
Antioxidant pomegranate studies

<table>
<thead>
<tr>
<th>Assay(s)</th>
<th>Assay type</th>
<th>Effect</th>
<th><em>Punica granatum</em> PART</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Carotene</td>
<td><em>In vitro</em> antioxidant</td>
<td>Inhibits twice as strongly as similar extract of red wine, slightly less than green tea</td>
<td>Fermented juice (W)</td>
<td>Organically grown source of W, red wine and green tea of extra fine quality</td>
<td>Schubert et al. (1999)</td>
</tr>
<tr>
<td>ABTS, DMPD, FRAP</td>
<td><em>In vitro</em> chemical antioxidant</td>
<td>Three times stronger inhibition that red wine or green tea, significantly stronger than aril-pressed juice</td>
<td>Commercial PJ (whole fruit squeezed)</td>
<td>Commercial PJ is pressed from whole fruit with peels, contains considerably more punicalagin than aril-pressed PJ</td>
<td>Gil et al. (2000)</td>
</tr>
<tr>
<td>DPPH</td>
<td><em>In silico</em> antioxidant</td>
<td>Three times stronger inhibition that red wine or green tea, significantly stronger than aril-pressed juice</td>
<td>Commercial PJ (whole fruit squeezed)</td>
<td>Commercial PJ is pressed from whole fruit with peels, contains considerably more punicalagin than aril-pressed PJ</td>
<td>Gil et al. (2000)</td>
</tr>
<tr>
<td>Low density lipoprotein (LDL)</td>
<td><em>In vitro</em> biological antioxidant</td>
<td>Methanol extracts more potent inhibition than ethyl acetate or water extracts</td>
<td>Peels</td>
<td>Similar effects in chemical assays</td>
<td>Singh et al. (2002)</td>
</tr>
<tr>
<td>Low density lipoprotein (LDL)</td>
<td><em>In vitro</em> biological antioxidant</td>
<td>Methanol extract 1/4 as potent as same from peels</td>
<td>Seeds</td>
<td>Similar effects in chemical assays</td>
<td>Singh et al. (2002)</td>
</tr>
<tr>
<td>TEAC, inhibition of peroxidation of phosphatidylcholine liposomes</td>
<td>Chemical <em>in vitro</em> antioxidant</td>
<td>Prodelphinidin dimers potent antioxidants in aqueous phase, only gallocatechin-(4-8)-catechin more effective than prodelphinidin monomers in lipid phase (in liposomes)</td>
<td>Gallocatechin and prodelphinidins from peel</td>
<td></td>
<td>Plumb et al. (2002)</td>
</tr>
<tr>
<td>H₂O₂-induced LDL oxidation</td>
<td><em>In vitro</em> rat brain</td>
<td>Seventy percent acetonitrile extract and principle anthocyanidins inhibit, pelargonidin 25X more potent than delphinidin or cyaniding</td>
<td>Whole pomegranate fruit (WPFE)</td>
<td>Scavenging of nitric oxide (NO) not observed</td>
<td>Noda et al. (2002)</td>
</tr>
<tr>
<td>Tumor necrosis factor alpha (TNF-α) in vascular endothelial cells</td>
<td>Biological <em>in vitro</em></td>
<td>Inhibition</td>
<td>Fermented juice extract (W)</td>
<td>Suggests redox triggering of inflammation</td>
<td>Schubert et al. (2002)</td>
</tr>
<tr>
<td>Shear-stress-mediated-NF-κB activation in vascular endothelial cells</td>
<td>Biological <em>in vitro</em></td>
<td>Activation</td>
<td>Fermented juice extract (W)</td>
<td>Suggests redox triggering of inflammation</td>
<td>Schubert et al. (2002)</td>
</tr>
<tr>
<td>Shear stress in cultured human coronary artery endothelial cells</td>
<td>Biological <em>in vitro</em></td>
<td>Oxidation sensitive responsive genes down-regulated, endothelial NO synthase (eNOS) expression increased</td>
<td>Fresh juice (PJ)</td>
<td></td>
<td>de Nigris et al. (2005)</td>
</tr>
<tr>
<td>Low-density lipoprotein (oxLDL) induced human coronary endothelial cells</td>
<td>Biological <em>in vitro</em></td>
<td>Reverses downregulated endothelial nitric oxide synthase (NOSIII) expression; reduces NOSIII-mediated basal and bradykinin-stimulated cellular cGMP accumulation</td>
<td>Fresh juice (PJ)</td>
<td>NOSIII-mediated basal and bradykinin-stimulated cellular cGMP accumulation associated with athero-genesis and clinical vascular sequelae</td>
<td>de Nigris et al. (2006)</td>
</tr>
<tr>
<td>LDL oxidation and atherosclerotic plaque formation</td>
<td>Mice <em>in vivo</em></td>
<td>Prevents these effects, concentrating polyphenols and increasing paroxinase in macrophages</td>
<td>Fresh juice (PJ)</td>
<td></td>
<td>Aviram et al. (2002)</td>
</tr>
</tbody>
</table>
Biochemical assessments of oxidation

Rat in vivo, heart, liver, kidney

Reduces malonaldehyde, hyperoxides, and conjugated dienes; enhances glutathione

Ethyl acetate and methanol/ethyl acetate WPFE’s

Glutathione is an endogenous antioxidant, in response to oxidative threat

Sudheesh and Vijayalakshmi (2005)

Resistance of LDL to oxidation

Mouse in vivo, human serum ex vivo

Enhances resistance

PJ

Aviram et al. (2000)

Measurement of macrophage lipid peroxides and glutathione

Biological in vitro antioxidant

Three times more enhanced than treatment by PJ

Enzymatically treated, finely milled, pomegranate by-product (PBP), remains after conventional juicing

Rosenblat et al. (2006)

Paraoxonase-2 lactonase

Biological in vitro antioxidant

Significantly increased action

Enzymatically treated, finely milled, pomegranate by-product (PBP), remains after conventional juicing

Rosenblat et al. (2006)

Lipid and TEAC (Trolox equivalent antioxidant capacity)

Chemical in vitro antioxidant

Antioxidant actions as PJ > TPT > punicalagin > EA

PJ, total pomegranate tannins (TPT), punicalagin, and ellagic acid (EA)

Suggest synergistic actions among PJ compounds

Seeram et al. (2005)

DPPH

Chemical in silico antioxidant

Scavenges reactive species (ROS) superoxide (O$_2^-$*), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radicals (OH)

Ethanolic pomegranate flower extract (PFE)

Unlike PJ and its anthocyanins (Noda et al., 2002), also scavenges NO, also abrogates liver toxicity (see text)

Kaur et al. (2006)

DPPH

Chemical in silico antioxidant

Punica granatum leaves strongest antioxidant (93.5% enhancement) of almost 100 species tested

Leaf extract

0.5 mg/ml, incubated at 37 °C 20 min. minutes, second place Acer buergerianum Miq., 86.4%

Lu et al. (2003)

Endothelial nitric oxide synthase (NOSIII) induction by oxidized LDL in human coronary endothelial cells

Cellular system in vitro

Reversed potent down-regulation of NOSIII induced by ox-LDL

PJ

Suggests beneficial effects on vascular complications by enhancing NOSIII bioactivity

Ignarro et al. (2006)

DPPH, luminol/xanthine/xanthineoxidase

In silico, chemilumi-nescense in vitro

Good antioxidant activity, strongest from EA extract of arils

Aris, PJ, peels and their EA extracts

Ricci et al. (2006)
prostate cancer cells in G2/M (P < 0.05) were effected by PSO, W, and P, but only P and W and not PSO decreased the percentage of cells in G1. These results, however, are not universal in all androgen negative human prostate cell lines. For example, effects of these pomegranate components are less pronounced or not seen in PC-3 human prostate cancer cells exposed to the same agents (Albrecht et al., 2004).

4.3. Differentiation

Differentiation of HL-60 promyelocytic human leukemia cells as detected by nitro blue tetrazolium reducing, non-specific esterase, specific esterase and phagocytic activities is potently promoted by P and W, whereas the ethyl acetate extract of fresh, unfermented pomegranate juice (J) has little effect. Differentiation may possibly figure into observed anticancer effects of pomegranate extracts in other cell lines, including breast and prostate (Kawai and Lansky, 2004).

4.4. Other enzymes

Carboxic anhydrase (CA) catalyzes the reversible hydration of carbon dioxide (CO2) to bicarbonate (HCO3−) (Khalifah, 2003). The presence of CA in every fluid compartment along the pathway of CO2 transport enables hydration of HCO3− to coordinate with the rapid diffusion of CO2 across biological membranes (Henry and Swenson, 2000). At least 14 isoforms of CA are known in mammals, and though the mechanism is not known, many CA inhibitors (CAI’s) strongly inhibit cancer cell growth in vitro and in vivo. Thus, CAI’s may therefore provide novel anti-tumor drugs (Pastorekova et al., 2004). Since CA’s also possess esterase activity, it is of interest that pomegranate peel extract and some of its active ellagitannins inhibit the de-esterification of p-nitrophenyl acetate catalyzed by CA’s, establishing their activity and role as CAI’s (Satomi et al., 1993) and raising the possibility of CA inhibition as another aspect of pomegranate’s anticancer actions.

Ornithine decarboxylase (ODC) catalyzes formation of polyamines such as the naturally occurring putrescine, spermidine and spermine from the amino acid ornithine, itself a product of the breakdown of l-arginine by arginase to form urea. Polyamines regulate growth processes in both eukaryotic and prokaryotic cells, and may facilitate the growth of cancer (Bachrah, 2004). Pomegranate may also serve as a source of inhibitory components to inhibit this enzyme since epidermal ODC is inhibited by PSO (Hora et al., 2003) as well as by acetone WPFE’s (Afaq et al., 2005b).

Aromatase, estrogen synthase, catalyzes the formation of estrone and estradiol from androstenedione and testosterone, respectively (Karaer et al., 2004). As the rate limiting enzyme in endogenous estrogen synthesis, aromatase can promote hormone dependent cancers, so its inhibition is an important therapeutic objective for certain malignancies, e.g., estrogen sensitive breast cancers. It is therefore significant that aromatase is potently inhibited by both W and P, although only weakly inhibited by PSO (Kim et al., 2002).

17-β-Hydroxysteroid dehydrogenase type 1 catalyzes reduction of estrone to the much more potent estrogenic 17-β-estradiol. Since this reaction also increases estrogenic stimulation of estrogen sensitive cancers, this enzyme is a valid target of importance for controlling malignant breast disease. In recognition of this, supercritical CO2 extracted PSO (SESCO) much more potently inhibits 17-β-hydroxysteroid dehydrogenase type 1 than does either W or P (Kim et al., 2002).

A note of caution should be inserted here concerning the multiple enzyme systems shown to be influenced by specific pomegranate fractions. These findings would be more compelling against a background of negative influence of enzymes not relevant to the present discussion. Unfortunately, in many cases investigators have simply not looked at relative specificity of biochemical effects with respect to changes in normal and malignant cells or tissues. A much wider future screening of enzymes and their possible modulation (or lack of it) by pomegranate fractions is thus called for.

5. Cancer treatment

5.1. Angiogenesis

The initiation and development of new blood vessels (angiogenesis) are essential to supply oxygen and nutrients for tumor growth and metastasis. Inhibition of tumor blood vessel formation, first proposed by Judah Folkman in the early 1970s (Folkman, 1972), is still a relatively non-toxic and promising therapeutic approach for treating solid tumor afflicted patients. Interestingly, recent research indicates that pomegranates possess the ability to inhibit development of new blood vessels. Thus, angiogenesis in chicken chorioallantoic membrane (CAM) in vivo was significantly suppressed by W but not by P. Pro-angiogenic vascular endothelial growth factor (VEGF) was potently downregulated in MCF-7 estrogen dependent breast cancer cells, less so in estrogen negative MDA-MB-231 breast cancer cells, and most strongly in MCF-10A immortalized normal breast epithelial cells by W and SESCO, and only mildly so by P and pressed PSO. The anti-angiogenic migration inhibitory factor (MIF) was potently upregulated in MDA-MB-231 cells by W and SESCO, which also moderately suppressed human umbilical vein endothelial cell (HUVEC) proliferation and tubule formation. Conversely, P and W (SESCO and PSO only weakly so) potently inhibited human myometrial fibroblast proliferation (Toi et al., 2003) suggesting selectivity to the inhibition of cellular proliferation amongst cell types.

5.2. Apoptosis

Apoptosis, an early response cell death, is a useful marker for predicting tumor response after anticancer treatment. Aqueous pomegranate peel extract resulted in apoptotic DNA fragmentation and suppression of growth in two human Burkitt’s lymphoma cell lines, Raji and P3HR-1 (Settheetham and Ishida, 1995) while 50 μg/ml PSO led to 54% greater extent of apop-
tosis in MDA-MB-435 estrogen receptor negative, metastatic human breast cancer cells, compared to the known apoptotic compound, Δ-tocopherol (Kim et al., 2002). Pomegranate fractions have also been shown to result in apoptosis in two androgen receptor negative human prostate cancer cell lines in the highly metastatic PC-3 (P=W>PSO), and in the slower growing DU-145 (PSO>P=W). These activities were at least partially mediated by caspase enzymes (Albrecht et al., 2004), suggesting involvement of inflammatory processes in executing the suicidal apoptotic cascades (Johar et al., 2004). Caspase activation in PC-3 cells by WPFE correlates with downregulation of pro-apoptotic factors Bax and Bak and downregulation of anti-apoptotic factors Bcl-XL and Bcl-2. Similarly, WPFE reduced expression of cyclins D1, D2, and E and cyclin-dependent kinase (cdk) 2, cdk4, and cdk6, while PJ or TPT, standardized to 100 μg punicalagin, or 100 μg EA or 100 μg punicalagin, each effected apoptosis in HT-29 human colon cancer cells, but only TPT, punicalagin and EA induced apoptosis in HCT116 colon cancer cells, suggesting PJ contains anti-apoptotic factors as well as pro-apoptotic ones (Seeram et al., 2005). In Caco-2 human colon cancer cells but not CCD-112CoN normal human colon cells, both punicalagin and its hydrolysis product EA down-regulated cyclins A and B1 and upregulated cyclin E resulting in cell-cycle arrest in S phase, and apoptosis via an intrinsic pathway (FAS-independent, caspase 8-independent) through Bcl-XL down-regulation with mitochondrial release of cytochrome c into the cytosol and activation of initiator caspase 9 and effector caspase 3, suggesting that these effects of punicalagin are mediated mainly or entirely via EA (Larrosa et al., 2005). Thus in short, both the lipid and aqueous pomegranate fractions appear to possess selective apoptotic potential in respect to different hormone-independent cancer cell lines, suggesting chemotherapeutic potential for compounds originating from each of these pomegranate compartments.

5.3. Tumor cell invasion

Approximately 90% of all cancer deaths arise from the metastatic spread of primary tumours. Of all the processes involved in carcinogenesis, local invasion and the formation of metastases are clinically the most relevant, but they are the least well understood and have been amongst those processes most difficult to target. Nonetheless, recent research has indicated that pomegranate appears to contain components capable of suppressing tumor cell invasion. Cold-pressed PSO, for example, inhibited invasion of estrogen sensitive MCF-7 human breast cancer cells in vitro across an artificial Matrigel™ membrane at doses less than 10 μg/ml (Kim et al., 2002), and PSO, P, and W each resulted in 60% suppression of invasion in Matrigel™ of human PC-3 androgen negative prostate cancer cells at 3 μg/ml (Albrecht et al., 2004). When equal amounts of any two of W, P or PSO were combined as 1.5 + 1.5 = 3 μg/ml, a supra-additive, synergistic effect was obtained such that the combination resulted in a 90% suppression of invasion. When all three were combined as 1 + 1 + 1 = 3 μg/ml, the suppression exceeded 99% (P<0.01) by Kruskal–Wallis non-parametric H-test (Lansky et al., 2005a). At 1 μg/ml, pure punicic acid inhibited PC-3 invasion 70%, luteolin 60%, EA 60%, and caffeic acid 50%. Any two combined effected a non-significant enhanced suppression, but punicic acid, luteolin and caffeic acid together resulted in a statistically significant 95% suppression, while addition of EA to the mix weakened the apparent synergy (Lansky et al., 2005b).

5.4. Proliferation

The ability of any chemotherapeutic agent to inhibit selectively proliferation of malignant but not normal cells is the hallmark of a promising anticancer therapeutic agent. In this regard, pomegranate peel extracts have been shown to retard proliferation of cells in several different human cancer cell lines (Settheetham and Ishida, 1995; Mavlyanov et al., 1997; Kawai and Lansky, 2004). In human breast cancer cells, for example, the effects of W and P were most pronounced against estrogen responsive MCF-7 cells, less pronounced against estrogen negative MDA-MB-231 cells, and least pronounced against immortalized normal breast epithelial cells MCF-10A (Kim et al., 2002; Toi et al., 2003) strongly suggesting a spectrum of anticancer activity and not the presence of indiscriminate cytotoxic compounds. Also, additive inhibition of proliferation by the isoflavone genistein, common in soy, clover and other legumes, and W occurred in MCF-7 cells, but further studies are required to determine if the additive effect is also supra-additive (Jeune et al., 2005). In human prostate cancer cells, DU-145 androgen independent cells were more sensitive to W and P than to cold-pressed PSO, the effect milder in PC-3 androgen independent cells or LNCaP androgen sensitive cells, but LNCaP cells were most sensitive to PSO relative to W or P. Notably, immortalized normal prostate epithelial cells hPrEC were found to be considerably less affected by either W or P than were androgen sensitive cancer cells LNCaP (Albrecht et al., 2004). When sub-lethal doses of P (6.25 μg/ml) or PSO (16.25 μg/ml) were combined with an anti-proliferative dose of W (25 μg/ml), supra-additive enhancement of the suppression of proliferation ensued (P<0.001) (Lansky et al., 2005a). Similarly, the anti-proliferative actions of a TPT, punicalagin or EA were less potent against human oral (KB, CAL27), colon (HT-29, HCT116, SW480, SW620) or prostate (RWPE-1, 22Rv1) cancer cells than PJ standardized to the same dose of punicalagin used as a single agent (Seeram et al., 2005). Treatment of androgen-independent PC-3 cells with acetone WPFE dose-dependently inhibited proliferation, corresponding to changes in the cyclin kinase inhibitor-cyclin-cdk network, and WPFE treatment of nude mice implanted with androgen-sensitive CWR22Rnu1 human prostate cancer cells resulted in suppression of growth and a significant decrease in serum prostate-specific antigen (Malik et al., 2005). These studies collectively reinforce the hypothesis that whole, complex pomegranate products possess potential anti-proliferative activity against cancer cells superior to that of their key active compounds, again, suggesting therapeutic strategies that may depart from the traditional preference for pure single agents.
5.5. Contribution of pomegranate components with estrogenic activity

We have previously shown that pomegranate components possess an ability to inhibit the estrogenic action of 17β-estradiol, an activity best explained through competitive binding to estrogen receptors by a number of non-steroidal estrogenic flavonoids such as kaempferol, quercetin, naringenin, and luteolin (Kim et al., 2002). A methanolic eluate of pomegranate juice competed with 17β-estradiol for estrogen receptors, stimulated estrogen receptor positive (ER+), MCF-7 breast cancer cells and increased uterine weight in ovariectomized mice (Maru et al., 2001), though elsewhere, pomegranate fermented juice inhibited MCF-7 growth through a range of concentrations (Kim et al., 2002). Further, an estrogen-agonist protective effect of pomegranate juice and seed extract was shown in ovariectomized mice, evidenced by improved bone density and attenuation of experimental depression (Mori-Oakamoto et al., 2004), and both oral and intramuscularly injected pomegranate seed oil increased uterine weight and vaginal cornification in ovariectomized mice and immature female rabbits, respectively (Sharaf and Nigm, 1964; Sharaf, 1969). Estrogenic agonism may exert cancer therapeutic potential by inhibiting androgenic activity, especially in prostate cancer (Zhu et al., 2005), but also by anti-inflammatory mechanisms (Harris et al., 2003; Harris, 2006) or by promoting apoptosis in cancer cells via Fas/FasL pathways (Song and Santen, 2003).

5.6. Phase 2 clinical trial

Despite the impressive amount of preclinical work indicating cancer preventive or therapeutic efficacy with limited toxicity, there still remain few well designed clinical trials investigating the relative anticancer health benefits of pomegranate. Against this paucity of clinical work, in a recent open-label, single-arm, 2-year, phase-2, Simon two-stage clinical trial of 48 men (46 men actually completed the trial) with increasing prostate-specific antigen (PSA), an important surrogate biomarker for prostate cancer mortality after surgery or radiotherapy, 8 oz daily of pomegranate juice (wonderful variety, 570 mg, total polyphenol gallic acid equivalents) per orum resulted in a significant increase in PSA doubling time from a mean of 15–37 months (P = 0.048). Furthermore, ex vivo application of the post-treatment patient serum to LNCaP androgen-sensitive cancer cells in culture resulted in a 12% decrease in cell proliferation and a 17% increase in apoptosis (P = 0.0048 and 0.0004, respectively), 23% increase in serum nitric oxide (P = 0.0085), and significant (P < 0.02) reductions in oxidative state and sensitivity to oxidation of serum lipids after versus before pomegranate juice consumption. Eligible patients had a detectable PSA > 0.2 and < 5 nm/L that was documented as rising, enough pretreatment PSA time points to calculate a baseline PSA doubling time (PSADT), no hormonal therapy before entering the study, no evidence of metastatic disease, and Gleason score ≤ 7. Markers for compliance included serum and urinary polyphenol/ellagic acid levels. Though the careful study design exacted significance, it suffered from a lack of placebo groups. No serious adverse effects were reported in any of the participants (Pantuck et al., 2006a,b).

6. Toxicology

Pomegranate has been widely consumed by persons in many different cultures for thousands of years, largely without untoward incident, and thus is considered generally safe. However, some toxicity is known, and undoubtedly, more remains to be discovered. Consumption of decoction of the tree bark, and to a lesser extent, pericarps of the fruit, may cause severe acute gastric inflammation and even death due to the presence of both tannins and alkaloids (Squillacci and Di Maggio, 1946). Whole fruit extracts have been shown to cause congestion of internal organs and elevated creatinine in vivo (Vidal et al., 2003). Pomegranate seed oil was not toxic to brine shrimp larvae (Fatope et al., 2002), however both severe allergic reactions (Igea et al., 1991; Gaig et al., 1999; Hegde et al., 2002) from eating the fruit and esophageal cancer from chronic consumption of roughly ground pomegranate seeds (Ghadirian, 1987; Ghadirian et al., 1992) have been reported.

7. Conclusion

Pomegranate is an ancient fruit with an illustrious medical history and has been the subject of classical reviews for over 100 years (Lloyd, 1897; Li et al., 2002). However, until only very recently, the importance of the oily phase of the seed has been largely overlooked. Recent studies have also begun to suggest possible synergistic interactions between aqueous and lipid phases of the fruit, and between different chemicals in each phase. Though, undoubtedly, much more is still unknown than known about the pomegranate’s chemistry and medicinal potential, the beginnings of a possible use for the fruit in cancer chemoprevention (Malik and Mukhtar, 2006) and chemotherapy, largely deriving from the anti-inflammatory properties of both the aqueous and lipid phases, is in the earliest stages of being appreciated (Longtin, 2003). Clinical trials with pomegranate materials, though, particularly with regard to inflammation and cancer, are still sorely lacking.

Much of the work completed on pomegranate over the past 7 years has focused on antioxidant activity of the tree’s various components. The relationship of this activity to health and disease has not been established, so direct extrapolation of such findings to medical recommendations is premature. In short, the studies reported in this survey while possibly provocative, leave many gaps. Though inconclusive, however, they do suggest further study, including clinical trials of properly designed pharmaceutical products. Toward such an end, it is hoped that the present review will provide some valuable clues for ongoing explorations of this most fascinating botanical species. Note: At the time of resubmission of this manuscript, the authors have learned of a new medical monograph on the subject of Punica granatum (Seeram et al., 2006), and also an editorial on the subject of pomegranate—pharmaceutical interactions (Summers, 2006). It is likely that much more will follow, as the medical community and public continue to...
exhibit renewed interest in the pomegranate as a therapeutic article.

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